positive inside rule. Even though there are many similarities the overall distributions are remarkably different between alpha and beta proteins, with beta-barrels displaying a much narrower hydrophobic core. We confirm that hydrophobic residues are the main driving force behind membrane protein insertion, while polar, charged and aromatic residues were found to be important for the correct orientation of the helix inside the membrane.

2407-Pos Board B177
Biophysical Characterization of the FtsL/B Subcomplex of the Bacterial Divisom
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Cell division in the gram-negative bacterium *Escherichia coli* is carried out through the cooperative assembly of at least 10 required proteins, known as the divisome. Few divisome proteins have described functions, and the structural details of their interactions are still primitive. The focus of this study is the structural characterization of two proteins that interact independent of other divisome components; FtsL and FtsB. Both proteins contain a small cytoplasmic tail, a single-pass transmembrane domain (TMD), and a predicted coiled-coil domain (CCD). We are also structurally characterizing the homologs of FtsL and FtsB, FtsL and DivIC, respectively, in *Bacillus subtilis,* the gram-positive model system of bacteria.The sequences of FtsL and FtsB are not highly conserved, but similar proteins are present in other bacterial species. FtsL and FtsB rapidly degrade in the absence of one another *in vivo*, so their interaction is a possible regulation point in division. Additionally, the stability of FtsL and DivIC is predicted to be important for guarding against transmembrane proteolysis. Here, we present a detailed structural characterization done through the use of use of *in vitro* biophysical interaction assays, *in vivo* screening methods, and computational modeling. Due to the difficult nature of studying integral membrane proteins, we have dissected FtsL and FtsB into their separate domains and performed biophysical interaction assays to determine how the domains work together to interact *in vitro*. Our combinatorial approach will provide a basis for functional hypotheses of these proteins *in vivo*, significantly improving our understanding of bacterial cell division.

2408-Pos Board B178
Structure and Assembly of *Escherichia Coli* Outer Membrane Protein A
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Outer membrane protein A (OmpA) of *Escherichia coli* is a paradigm for the biogenesis of outer membrane proteins; however, the structure and assembly of OmpA remain controversial. Studies to date support the hypothesis that native OmpA is a single-domain large pore, and a two-domain narrow pore conformer is a folding intermediate. The *in vitro* refolding of OmpA to the large pore conformation requires that the protein be isolated from outer membranes with an intact disulfide bond and then adequately incubated in lipids at temperatures $\geq$ 26 °C to overcome the energy of activation for refolding. The *in vivo* maturation of the protein involves covalent modification of serines of the N-terminal domain by oligo-(R)-3-hydroxybutyrates as the protein is escorted across the cytoplasm by SecB for post-translational secretion across the SEC translocase in the inner membrane. After cleavage of the signal sequence, protein chaperones, such a Skp, DegP and SurA, guide OmpA across the periplasm to the BAM complex in the outer membrane. During this passage, a disulfide bond is formed between C290 and C302 by DsbA, and the hydrophobicity of segments of the C-terminal domain which are destined for incorporation as β-barrels in the outer membrane bilayer is increased by covalent attachment of oligo-(R)-3-hydroxybutyrates. With the aid of the BAM complex, OmpA is then assembled into the outer membrane as a single-domain large pore.

2409-Pos Board B179
A Structural Recognition Mechanism Study of MARCH and its Substrate
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Ubiquitination, used by all eukaryotic cells to tag proteins for proteasomal degradation, is also involved in membrane protein regulation. The process of ubiquitination is mediated by ubiquitin ligases, such as the MARCH ligases in this study. The MARCH ligases are membrane proteins and play a major immunoregulatory role in cells of the immune system. They recognize their (membrane protein) substrates via transmembrane (TM) interactions_a poorly understood phenomenon. In order to characterize these TM-induced MARCH-substrate interactions, we have performed replica exchange molecular dynamics (REX-MD) simulations in an implicit membrane to model TM structures of MARCH-1 and MARCH-9 based on their TM and loop sequences. Both MARCHs have two putative TM helices (TM1 and TM2) connected by an extracellular loop. The key TM1-TM2 interfacial residues and the relative orientation of the two TMs are identified and presented. We have also determined possible TM interaction modes of known MARCH-substrate by REX-MD simulations of the complex structure, and the results will be presented in terms of the molecular basis of the MARCH-substrate recognition mechanisms.

Protein Biophysics in vivo

2410-Pos Board B180
Folding Dynamics of Trp-Cage in the Presence of Chemical Interference and Macromolecular Crowding
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Proteins fold and function in the crowded environment of the cell's interior. In the recent years it has been well established that the so-called ''macromolecular crowding'' effect enhances the folding stability of proteins by destabilizing their unfolded states for selected proteins. On the other hand, chemical and thermal denaturation is often used in experiments as a tool to destabilize a protein by populating the unfolded states when probing its folding landscape and thermodynamic properties. However, little is known about the complicated effects of these synergistic perturbations acting on the kinetic properties of proteins, particularly when large structural fluctuations, such as protein folding, have been involved. In this study, we have first investigated the folding mechanism of Trp-cage dependent on urea concentration by coarse-grained molecular simulations where the impact of urea is implemented into an energy function of the side chain and/or backbone interactions derived from the all-atomistic molecular dynamics simulations with urea through a Boltzmann inversion method. In urea solution, the folding rates of a model mini protein Trp-cage decrease and the folded state slightly swells due to a lack of contact formation between side chains at the terminal regions. In addition, the equilibrium m-values of Trp-cage from the computer simulations are in agreement with experimental measurements. We have further investigated the combined effects of urea denaturation and macromolecular crowding on Trp-cage's folding mechanism where crowding agents are modeled as hard-spheres. The enhancement of folding rates of Trp-cage is most pronounced by macromolecular crowding effect when the extended conformations of Trp-cast dominate at high urea concentration. Our study makes quantitatively testable predictions on protein folding dynamics in a complex environment involving both chemical denaturation and macromolecular crowding effects.

2411-Pos Board B181
Protein-Protein Interactions in Crowded Cellular Environments
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Explicit solvent fully atomistic molecular dynamics simulations of chymotrypsin 2 in concentrated protein solutions are described to better understand the role of protein-protein interactions in crowded cellular environments. The effect of protein crowding on protein stability and self-diffusion is discussed and compared with experimental data. An energetic analysis is presented to quantitatively analyze the components of the free energy of crowding, i.e. the transfer free energy between dilute solvent and crowded environments.

2412-Pos Board B182
Quantitative Theory for Protein-Protein Interactions in a Crowded Environment
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Protein-protein interactions play an essential role in many biological processes, including DNA transcription regulation, signal transduction, membrane-protein trafficking and immune response. The cellular medium is crowded with an ensemble of macromolecules, e.g., proteins, nucleic acids, sugars and lipids. These macromolecules can occupy as high as 30% of the cell volume and interact with proteins via van der Waals and electrostatic interactions, thereby